Pharmacogenetics
Lessons from Anti Epileptic Drugs

David Goldstein
Center for Population Genomics and Pharmacogenetics
Institute for Genome Sciences & Policy
Duke University Medical Center

Carbamazepine and Phenytoin

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- Inexpensive
- Effective

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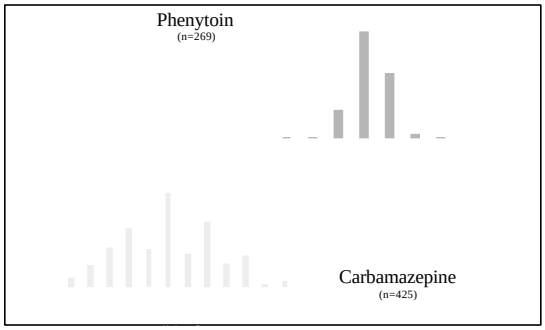
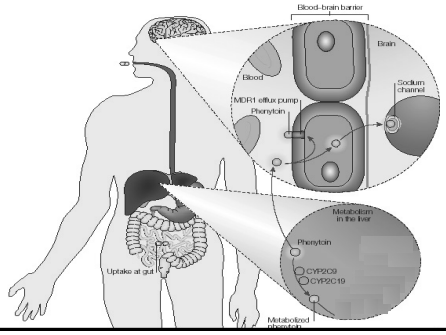
- **Appropriate doses, like with most AEDs and many other medicines, can take months to identify**

Maximum doses



Phenytoin Pathway



Strategy

Phenytoin
- Assess functional variants in CYP2C9
- Assess putative functional variant in ABCB1
- Assess tagging SNPs in SCN1A

Carbamazepine
- Assess putative functional variant in ABCB1
- Assess tagging SNPs in SCN1A

Tagging SNPs

to represent common (known & unknown) variation

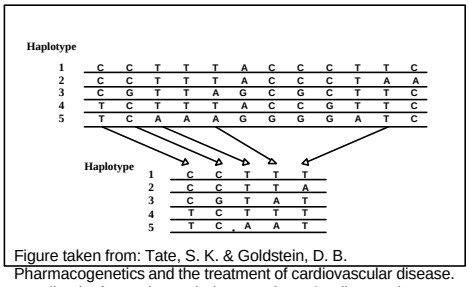


Figure taken from: Tate, S. K. & Goldstein, D. B. Pharmacogenetics and the treatment of cardiovascular disease. *Handbook of Experimental Pharmacology: Cardiovascular Pharmacogenetics* **160**: 25-37 (2004)

Tagging the major human DMEs

- **Approximately 200 SNPs are sufficient to represent the greater than 4,000 common polymorphisms in key genes regulating drug plasma levels**
- *(Ahmadi, Weale et al, 2005, Nature Genetics)*

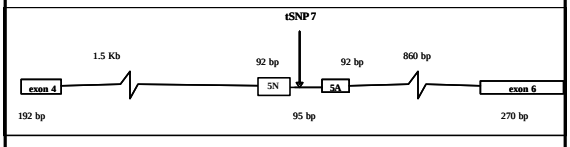
Results

CYP2C9 Allele	Freq. (%)	Average Phenytoin Dose (mg)			Sig.
		wt/wt	wt/var	var/var	
*2	11.9	357	339	317	0.38
*3	9.7	362	304	250	**0.005**

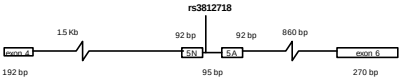
SCN1A – "tag 7"

	AA	AG	GG	Significance
Phenytoin	373	340	326	p=0.005
Carbamazepine	1312	1225	1083	p=0.001

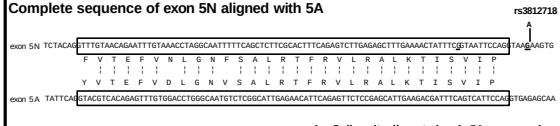
SCN1A tSNP7



Genomic structure of SCN1A around exon 5N and exon 5A



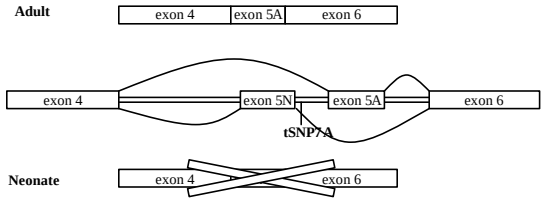
Complete sequence of exon 5N aligned with 5A



A = Splice site disrupted, only 5A expressed

G = Both 5N and 5A expressed

tSNP7 may affect SCN1A splicing in humans

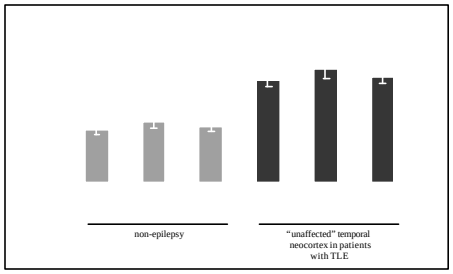


tSNP7 A = splice site disrupted, expression of exon 5N altered altered 5A/5N ratio?

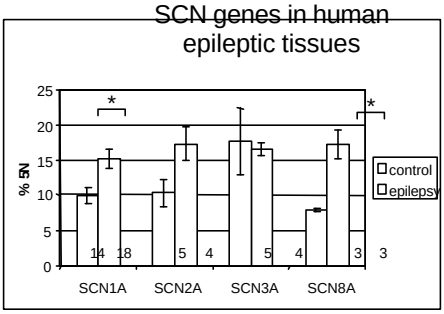
The proportion of 5N transcript is significantly higher in "unaffected" temporal lobe compared with seizure focus (hippocampus)

Genotype	"Un affected" temporal lobe	hippocampus	P value
AA	10.9	11.7	0.54
GG	14.6	11.2	0.02

Brain mRNA expression of SCN1A exon5A and 5N variants

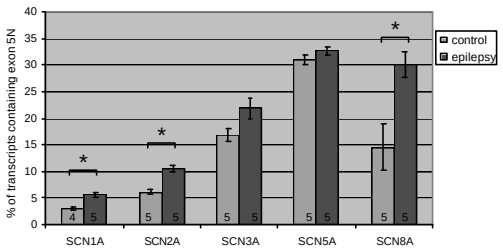


Exon 5N is up-regulated in SCN genes in human epileptic tissues



- Control RNA was purified from tissues in the Parkinson's Brain Bank
- For all but SCN1A, sample sizes are small
- SCN5A and SCN9A were not reliably detected in these tissues

In rats: exon 5N is widely up-regulated in models of epilepsy



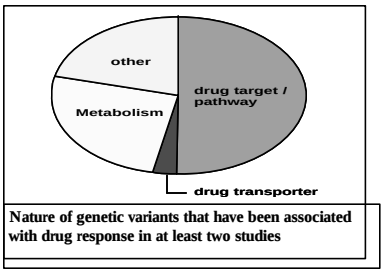
- Rats were 8 weeks old, tissues were harvested 3 weeks after pilocarpine injection
- SCN9A was not reliably detected in cortical tissues

Some good news…

- Haplotype tagging can identify unknown functional variants

Pharmacogenetics is a *simpler complex trait*

- Obvious candidate genes often carry gene variants that influence drug response
- Many of the causal variants are common



Nature of genetic variants that have been associated with drug response in at least two studies

Pharmacogenetics is a *simpler complex trait*

- Obvious candidate genes often carry gene variants that influence drug response
- Many of the causal variants are common
- <u>There is often the possibility of direct clinical relevance (change dose, select appropriate drug, etc)</u>

Pharmacogenetics is Transalational Research

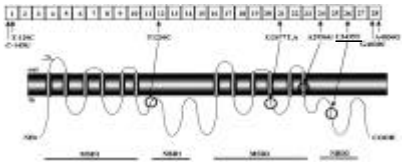
- 1) It is appropriate to prioritize projects based on potential clinical relevance
- 2) An argument can be made that a certain degree of exploratory pharmacogenetics (targets and target pathway, relevant DMEs, relevant transporters) should be a mandatory part of the drug development process

Refractory epilepsy

- ~30% of patients do not have their seizures controlled pharmacologically

ABCB1 encodes for the multidrug transporter protein PGP

- 28 exons, 209 Kb
- encodes PGP: 170 KDa, 12 transmembrane domains
- highly expressed in CNS, small intestine, colon, testis, placenta, liver, kidney, PBC
- key determinant of multidrug resistance and up-regulated in cancer cells



ABCB1 C3435T and refractory epilepsy

Siddiqui et al. 2003

Phenotype	CC	CT	TT
Drug-resistant (%)	55 (27.5)	106 (53.0)	39 (19.5)
Drug-responsive (%)	18 (15.7)	63 (54.7)	34 (29.6)
Control (%)	37 (18.5)	116 (58)	47 (23.5)

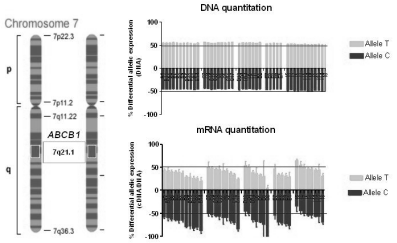
c^2 = 7.65, P = 0.006
OR: 2.66 (1.32 -5.38)

Tan et al. 2004

Phenotype	CC	CT	TT
Drug-resistant (%)	75 (18.7)	193 (48.1)	133 (33.2)
Drug-responsive (%)	37 (17.8)	115 (55.3)	56 (26.9)

P = 0.21

MDR1 allelic imbalance



Acknowledgments

- Epilepsy Genetics (Brussels)
  Chantal Depondt
- Tagging SNPs
  GSK, Mike Weale, Kourosh Ahmadi
- Epilepsy genetics London
  Sarah Tate
  Gianpierro Cavalleri
  Anna Need
  Stephanie Schorge
  Nicole Soranzo

  Asra Siddiqui
  Mike Weale
  Ley Sander
  Mari Wyn Burley
  Richard Marguerie
  Nicholas Wood
  Sanjay Sisodiya